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An antibody microarray, in multiwell plate format, for multiplex screening of foodborne pathogenic bacteria and biomolecules

Andrew G. Gehring · David M. Albin · Sue A. Reed · Shu-I Tu · Jeffrey D. Brewster

Abstract Intoxication and infection caused by foodborne pathogens are important problems worldwide, and screening tests for multiple pathogens are needed because foods may be contaminated with multiple pathogens and/or toxic metabolites. We developed a 96-well microplate, multiplex antibody microarray method to simultaneously capture and detect *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium (*S. typhimurium*), as well as a biomolecule (chicken immunoglobulin G or IgG employed as a proteinaceous toxin analog) in a single sample. Microarrayed spots of capture antibodies against the targeted analytes were printed within individual wells of streptavidin-coated polystyrene 96-multiwell microtiter plates and a sandwich assay with fluorescein- or Cy3-labeled reporter antibodies was used for detection. (Printing was achieved with a conventional microarray printing robot that was operated with custom-developed microplate array-ing software.) Detection of the IgG was realized from ca. 5 to 25 ng/mL, and detection of *E. coli* O157:H7 and *S. typhimurium* was realized from ca. 10^6 to 10^9 and ca. 10^7 to 10^9 cells/mL, respectively. Multiplex detection of the two bacteria and the IgG in buffer and in culture-enriched ground beef filtrate was established with a total assay (including detection) time of ca. 2.5 h. Detection of *S. typhimurium* was largely unaffected by high concentrations of the other bacteria and IgG as well as the ground beef filtrate, whereas a small decrease in response was observed for *E. coli* O157:H7. The multiwell plate, multiplex antibody microarray platform developed here demonstrates a powerful approach for high-throughput screening of large numbers of food samples for multiple pathogens and toxins.

Keywords Antibody microarray · Bacteria · Fluorescence · Immunoassay · Multiwell, microtiter plate · Multiplex

Introduction

Sickness caused by foodborne pathogens, such as *Escherichia coli* O157:H7, is an important problem in the USA [1]. This was recently observed by the multistate outbreak of *E. coli* O157:H7 associated with the consumption of spinach [2]. At least 183 people in 26 states became infected, and one person died, from eating spinach that was accidentally contaminated with *E. coli* O157:H7. In addition, the perceived threat of intentional contamination of foods with pathogens is a major concern. Therefore, considerable effort has been undertaken to develop specific, rapid methods for the detection of pathogens associated with foodborne outbreaks [3–9].

Screening tests that simultaneously detect the presence of multiple pathogens have been developed, as production facilities (e.g., dairy farms) are known reservoirs for several pathogens (such as *E. coli* O157:H7 and *Salmonella* spp. [10]). Tests with the capacity to screen for molecules of differing size and shape are preferred because analytes of interest vary greatly (i.e., whole bacteria versus toxins [11]). In addition, the very low incidence of contamination requires the screening of large numbers of samples for reliable monitoring of food safety. To meet these needs, specific multiplex polymerase chain reaction (PCR) assays

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have been developed [5, 12, 13]. However, these procedures are limited by the number of primer pairs that can be used under the same PCR conditions, and the size of the PCR products, which must differ enough to be clearly viewed on an agarose gel. Real-time multiplex PCR procedures have been developed to address some of the limitations often encountered with conventional multiplex PCR [11]. However, real-time multiplex PCR is limited by the number of fluorescent probes available for detection, or by limited differences in melting temperature (i.e., G + C content) during melting curve analyses [11]. Nucleic acid microarrays, which are coated glass slides with hundreds or thousands of small nucleic acid sequences in individually distributed, orthogonally arranged micron-diameter spots, overcome these limitations of PCR and pose enormous potential for pathogen screening [6]. Also, nucleic acid microarrays have increased sensitivity versus PCR and agarose gel detection [14]. Similarly, protein arrays, which employ spots or parallel printed stripes containing antibodies in place of nucleic acid sequences, are being developed for the detection of foodborne pathogens. Several examples of antibody arrays that show promise for the multiplex detection of bacterial cells and/or toxins in complex sample matrices (e.g., foods) have been developed [4, 15–18] as well as commercialized [19].

In this study, the development of a novel antibody microarray for the simultaneous detection of foodborne pathogens (*E. coli* O157:H7 and *Salmonella* spp.) and a biomolecule (chicken IgG) in a 96-multiwell microtiter plate platform is presented. Though microarrays are typically prepared on glass substrates, the present method employs less costly polystyrene multiwell plates with significantly larger sample volumes. Multiwell plate substrates were chosen since the format is conducive to the high-throughput screening of large numbers of samples. In the future these assays can incorporate automated plate handling, washing, and pipetting systems, as well as automated systems for antibody-coated paramagnetic particle or immunomagnetic bead concentration and cleanup of samples, to produce a fully automated, high-throughput screening platform. In addition, a new microarray printing program was created for this study so that a relatively inexpensive microarray printing robot could be adapted to microtiter plate substrates. This multiplex protein microarray format, performed in individual wells of a 96-multiwell plate, may be used to rapidly assess multiple food samples for the presence of a variety of pathogens whether they exist as whole cells, cell fragments, or metabolites (e.g., toxins). This work focuses on the challenges of: (a) generating reproducible antibody arrays in wells of streptavidin-coated polystyrene multiwell plates; (b) determining conditions under which proteins and whole cells can be simultaneously captured and detected; and (c)

collecting and analyzing data from the arrays. Future studies will focus on improving detection limits and extending this approach to a larger number of targets.

Experimental

Materials

Materials used in this research included the following: phosphate-buffered saline (PBS) tablets, glycerol, Tween 20, bovine serum albumin (BSA; fraction V), and TPP polystyrene clear, sterile, flat-bottomed 96-multiwell microtiter culture plates (used as microarray "source" plates) from Sigma-Aldrich (St. Louis, MO); biotinylated goat anti-*Escherichia coli* O157:H7 antibody (used as a capture antibody; 1 mg/mL prepared in PBS/glycerol 60:40), biotinylated goat anti-*Salmonella* antibody (anti-CSA-1, used as a capture antibody; 1 mg/mL prepared in PBS/glycerol 60:40), fluorescein-labeled goat anti-*E. coli* O157:H7 antibody (used as a reporter antibody; reconstituted to 0.5 mg/mL in nanopure water or nH$_2$O as suggested by the manufacturer), and fluorescein-labeled goat anti-*Salmonella* antibody (anti-CSA-1, used as a reporter antibody; reconstituted to 0.5 mg/mL nH$_2$O as suggested) from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD); biotinylated goat anti-chicken antibody (H&L, used as a capture antibody; 1 mg/mL prepared in PBS/glycerol 60:40), chicken immunoglobulin G or IgG (whole molecule, used as antigen; 10 mg/mL prepared in nH$_2$O as suggested), and Cy3-labeled goat anti-chicken antibody (H&L, used as a reporter antibody; 1 mg/mL in nH$_2$O as suggested) from Rockland (Gilbertsville, PA); black-walled, clear/transparent and flat-bottomed, streptavidin-coated polystyrene 96-multiwell microtiter plates preblocked with 0.3% BSA (used as "destination" plates or substrates in microarray printing; hence, immunoassays were conducted in these plates) from Greiner Bio-One North America Inc. (Monroe, NC); *E. coli* O157:H7 strain B1409 and *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) strain G8430 from Centers for Disease Control (Atlanta, GA); modified Brain Heart Infusion broth from Becton Dickinson (Sparks, MD); and stomacher bags from Fisher Scientific (Pittsburgh, PA). Ground beef was purchased from a local supermarket, and contained 85% lean tissue. Chemicals not mentioned above were of reagent grade.

Apparatus

Solutions were printed into 96-well microplate wells using a SpotBot® Personal Microarray Robot (protein version) that held a single, SMP3 printing pin (TeleChem International, Inc., Sunnyvale, CA). Fluorescent images of the

microarrayed microtiter plates were produced with an LS400 laser scanner from Tecan (Research Triangle Park, NC). Incubations with shaking were conducted with the innOva™ 4000 from New Brunswick Scientific (Edison, NJ). Stomaching was performed in a Stomacher 400 Lab System from Seward Medical Ltd. (London, UK). A Petroff–Hausser counting chamber from Thomas Scientific (Swedesboro, NJ) was used to enumerate bacterial cells.

Growth and enumeration of bacteria

One milliliter of frozen stationary phase *E. coli* O157:H7 or *S. typhimurium* G8430 was thawed and added to 10 mL of modified Brain Heart Infusion broth. This was incubated at 37 °C for 18 h with shaking at 160 rpm. Cultures were enumerated with a Petroff–Hausser counting chamber as described by Gehring et al. [20]. Filtered culture-enriched ground beef homogenate was prepared by adding 25 g of ground beef to a stomacher bag along with 225 mL of modified Brain Heart Infusion broth, stomaching the bag on the "normal" setting for 30 s, and incubating the bag with its contents overnight (18 h) at 37 °C with shaking (160 rpm). The material passing through the integral filter in the stomacher bag was collected and stored at 4 °C until being used within 4 h. We refer to this material as cultured ground beef filtrate (CGBF). (CGBF simulates a sample which has been enriched and contains high levels of endogenous microorganisms, commonly referred to as background flora, and metabolic byproducts.)

Antibody preparation and microarray printing

The biotinylated capture antibodies were reconstituted in 60% PBS/40% glycerol (v/v) in order to prevent evaporation of the droplets and maintain a hydrated state for the capture antibodies [21]. The capture antibody solution was further diluted tenfold with PBS containing 10 mM sucrose prior to use.

The software for array printing in multiwell microtiter plates was written in our laboratory and comprises ca. 7,000 lines of Java code. The PlateSetup application provided a graphical user interface for setting all parameters involved in spotting, including the selection of source plate wells (96- or 384-multiwell plate), destination plate wells (6-, 24-, or 96-multiwell plate), array dimensions, spot size, spot spacing, subarraying, print dwell times, and printing pin washing/drying times (see Appendix). The PlateSpotter application controlled the spotting robot through an RS-232 serial connection and provided automated spotting based on a parameter file generated by PlateSetup. PlateSpotter also provided a graphical user interface for manually controlling the robot for calibration and testing. The software is available from the authors on request.

Greiner destination plates, precoated with streptavidin and preblocked with BSA, were rinsed three times with nH$_2$O (ca. 250 µL/well per rinse) and allowed to air-dry prior to usage in order to remove the film/residue observed in the wells. At least 100 µL of thoroughly mixed capture antibody solution (0.1 mg/mL for the biotin-conjugated antibacterial antibodies as well as the anti-chicken IgG antibodies) was pipetted into separate wells of the TPP source plate on the microarray printer. Contact printing, using default wash and contact settings (with the exception of 1-s dwell times for preprinting and printing) for PlateSetup, was conducted with an SMP3 (100-µm spot diameter) pin, which delivered a volume of 0.7 nL per contact stroke. The pins were manually sonicated for 5 min in distilled H$_2$O after each daily printing routine. Subarray spots from different (when applicable) capture antibody sources were spaced 190 µm apart in a row and the subarrays, replicated seven times, were spaced 200 µm apart in columns for each well that was used. Each slide was visually examined after printing to ensure that a spot was printed with each pin stroke. Upon completion of printing (ca. 30–90 min), the spotted destination plates were subjected to static incubation at room temperature (RT) for 1 h prior to use.

Antibody microarray detection of bacteria and chicken IgG in multiwell plates

The procedures for conducting a fluorescent, sandwich immunoassay (Fig. 1) in the multiwell antibody microarray detection of chicken IgG and foodborne bacteria generally followed the one previously described for microarray slides [4] with several modifications. (As with microarray printing above, all immunoassay procedures and reagents were at RT.) Wells of the destination plate, preprinted with capture antibody, were washed by being filled with 200 µL PBST (PBS containing 0.05% Tween 20), immediately emptied by rapidly inverting the plate, and residual liquid was removed by striking the upside down plate onto a paper towel on the lab bench. This wash procedure was repeated once with PBST. Analyte (100 µL of samples containing chicken IgG, bacterial stock, and/or combinations thereof serially diluted in either PBS or CGBF) was then added, and each array was subjected to static incubation for 1 h to allow analyte capture. Bacterial solutions consisted of varying concentrations of either *E. coli* O157:H7 or *S. typhimurium* (0 and from 10^2 to 10^9 cells/mL), in PBS or in CGBF. Bacterial dilutions in CGBF contained the second bacteria at 10^8 cells/mL as well as chicken IgG (100 µg/mL). During the incubation for capture, the reporter antibody solutions were prepared (1:10 for fluorescein antibacterial antibody conjugates or 1:100 for Cy3 anti-chicken IgG antibody conjugate) with PBST plus 0.5% (w/v) BSA
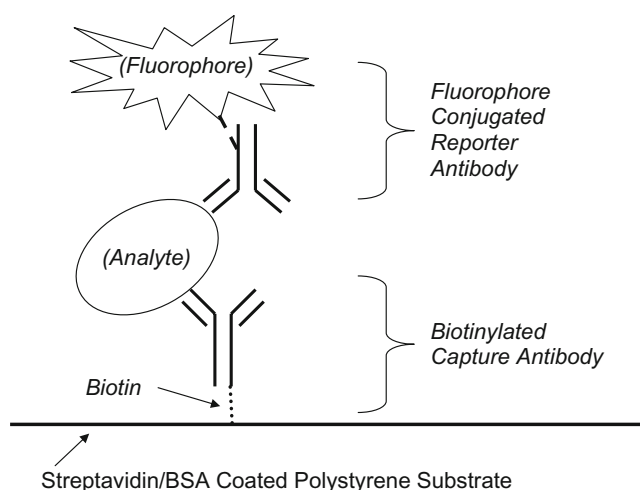
(Fluorophore)

Fluorophore
Conjugated
Reporter
Antibody

(Analyte)

Biotinylated
Capture Antibody

Biotin

Streptavidin/BSA Coated Polystyrene Substrate

Fig. 1 Schematic depiction of fluorescent sandwich immunoassay. Represented are analyte (e.g., *S. typhimurium* bacterial cell, antigenic cell fragment, or IgG) bound at a microarrayed spot of capture antibodies at the bottom of a single streptavidin-coated, BSA-blocked well of a multiwell, black-walled, clear-bottomed, polystyrene microplate. Biotinylated capture antibodies were contact printed onto the streptavidin surface using a microarray spotting robot. Following analyte capture, reporter antibodies, conjugated with fluorescent molecules, were used to sandwich and label the analyte in preparation for subsequent detection with fluorescence laser scanning

(PBST + BSA). The reporter antibodies were carefully protected from light during all experiments. The wells were washed twice with PBS + BSA and excess liquid was removed as above. Next, 100 μL reporter antibody solution was added to each well, which was subjected to static incubation for 1 h at RT. Wells were washed twice with PBST and dried as above, followed by two washes with PBS plus 10 mM sucrose (as above), and were then scanned at the appropriate fluorescence setting (fluorescein: excitation 488 nm, emission filter 535 nm; Cy3: excitation 543 nm, emission filter 590 nm) on the array scanner using either single-channel or dual-channel sequential scanning mode. Typical LS400 instrument scanning parameters, set and controlled via the Array-Pro Analyzer software (ver. 4.5.1.73) interface included autofocusing in well mode, PMT gain that ranged from 80–120, 20-μm resolution, small pinhole setting, and optimization of integration time = 1. (Scans of inverted multiwell plates were in a 6×1 format where two multiwell plate well columns were scanned at a time and final images were automatically "stitched" together and the image of the scanned plate was reoriented by the Array-Pro Analyzer software.)

Data analysis

Each well, which contained eight printed spots per analyte, was considered an experimental unit. Fluorescent images,

converted to 8-bit grayscale, were "Auto Levels" adjusted. The brightness and contrast levels were manually adjusted to optimize (by eye) signal to noise ratio in Adobe Photoshop (ver. 7.0). Fluorescent intensities (in fluorescence units or AFUs) of sample spot and local/proximal (adjacent to sample spots, or array of spots, in the same well) background pixels were obtained using an 8×2 grid of circles of identical size (9×9 pixels, width × height) with ScanAlyze software (ver. 2.50; Dr. Michael Eisen Laboratory, University of California at Berkeley). Net spot intensities (sample responses minus individual, corresponding background responses) were compared, and the two highest and two lowest values were discarded. Net intensities were then averaged and standard deviations were computed for the means.

Results and discussion

Challenges encountered with multiwell microarray analysis

Multiwell microtiter plates are well suited for the high-throughput screening of numerous food samples. The generation of microarrayed features (reaction sites), comprised of multiple antibodies, replicated in multiple microtiter plate wells allows for greater ease of sample manipulation and analysis than the traditional microscope slide-based, microarray substrate format. Multiwell plates accommodate much larger sample volumes, with the potential for proportionally greater sensitivity. Multiwell plates are also a less cumbersome format better suited to automated manipulation, but well surface area is at a premium relative to microscope slides. Fortunately, far fewer features are typically used in detection than in gene expression arrays. However, this study was not without aberrations that if not addressed in future research, will most likely result in failure of this approach for objective, automated sample analysis, especially if quantitative, as opposed to binomial (yes/no) results are desired (i.e., one of the problems encountered and presented below was the presence of fluorescing contaminants, e.g., dust, that if settled at a print location could produce false positive results that may confound automated, quantitative analysis).

Figure 2 displays a typical, contrast-enhanced fluorescent scan (actually a combination of images produced from scanning with blue and green lasers) of an inverted 96-multiwell microtiter plate that was used as a substrate for the antibody microarray. Plates had to be inverted prior to reading by the laser scanner due to limitations in instrumental focal length as well as potential production of side-wall reflections during image scanning. Several determinate and indeterminate errors contributed to random noise and
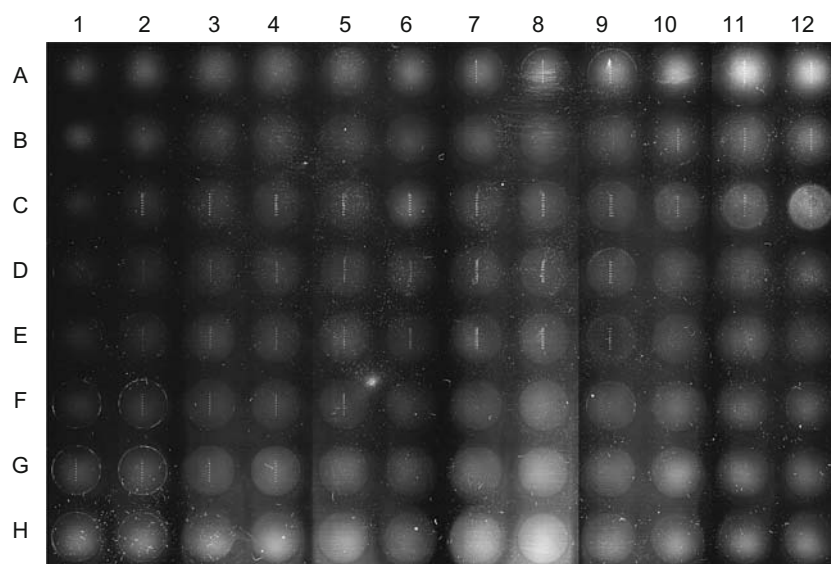
Fig. 2 Microarray laser scanning image of fluorescent sandwich immunoassay employed for the multiplex detection of *E. coli* O157: H7, *S. typhimurium*, and chicken IgG. The image was generated with a laser scanner used to read an inverted, 96-multiwell microtiter plate in six passes. This plate was used for multiple, simultaneous experiments (of which the results of some were compiled for presentation in this study) that involved the detection of various analytes including: *E. coli* O157:H7, 0 and 10^2–10^9 cells/mL in PBS (wells *A1–A9*, respectively), *S. typhimurium*, 0 and 10^2–10^9 cells/mL in PBS (wells *B1–B9*), *S. typhimurium*, 0 and 10^2–10^9 cells/mL in CGBF containing 10^8 cells/mL of *E. coli* O157:H7 and 100 ng chicken IgG (wells *C1–C9*), *E. coli* O157:H7, 0 and 10^2–10^9 cells/mL in CGBF containing 10^8 cells/mL of *S. typhimurium* and 100 ng chicken IgG (wells *D1–D9* and repeated in wells *E1–E9*), chicken IgG, 0, 6.25, 12.5, 25, 50, 100, 200, 400, and 800 μg/mL in PBS (wells *A10–A12*, *B10–B12*, and *C10–C12*) or in CGBF (wells *F1–F5* and *G1–G4*); The remaining wells were not exposed to any analytes

background fluorescence that limited the efficacy of sample spot analysis. Scratches, dust, fingerprints, light scattering, and apparently differential nonspecific binding of fluorescent antibody conjugate (perhaps due to variations in the well surfaces and/or inconsistent washing) were all sources for the noise and most instances can be readily observed in Fig. 2. Wiping the bottom of the plate with a lint-free cloth saturated with 70% ethanol followed by several blasts of canned air prior to scanning significantly reduced the incidence of these optical artifacts (data not shown), and washing the wells prior to spotting helped reduce background fluorescence variations.

Printing symmetrical and uniform spots devoid of smearing or "comet tails" is not trivial. The properties of the printing surface and the printing solution must be controlled to meet three potentially exclusive criteria. First, the solution must effectively wet the surface to allow binding. Second, spreading of the solution over the surface must be strictly limited to maintain small, discrete spots. Finally, the solution must maintain the biological integrity of the antibody. Although the printing process was not fully optimized, significant progress was made toward this goal by systematically studying several factors. It was found that rinsing and drying the destination plates before spotting appeared to remove a residual film of solids (perhaps salt, and/or excess streptavidin/BSA). This greatly improved

reproducibility and reduced clogging of the printing pin. Water was found to be as effective as buffer or detergent solutions for this rinsing step. Various concentrations of glycerol and biotinylated capture antibody in the spotting solutions were also evaluated. Glycerol was added to provide surface wetting, reduce spreading (by raising viscosity), and prevent drying and subsequent denaturation of the antibody. Satisfactory performance was found with 4% glycerol, and a relatively high concentration of antibody was selected to saturate the surface binding sites and provide uniform antibody coverage. However, although the spots were symmetrical and uniform prior to washing and imaging, the imaged spots often had "tails." This was attributed to nonuniform spreading of the viscous droplet as wash solution was added to the well. We investigated washing with biotin-containing solutions but this was not effective in reducing the incidence of smearing (data not shown). Regardless, spot symmetry was sufficient for accurate quantitation, but further improvement is expected.

Capture and detection of chicken IgG (a proteinaceous toxin analog)

Chicken immunoglobulin G (IgG) was used as an analog for a proteinaceous toxin in order to determine the efficacy of biomolecule capture and detection with the multiwell

Fig. 3 Fluorescent sandwich immunoassay in microarray format detection of chicken IgG. In **a**, the image was produced with a laser scanner by reading the microplate inverted. Shown is a single row of 12 wells containing increasing amount of IgG analyte from *left* to *right* exposed to each well printed with a single column of eight identical spots of biotinylated capture antibody. Response, in arbitrary fluorescent units (AFUs) was measured for increasing concentration of chicken IgG (from 0.39 to 400 ng/mL) in **b** buffer (PBS) and **c** CGBF. Aliquots of samples in a single dilution series were analyzed in quadruplicate. Each data point, four at each concentration level, represents eight spots or fluorescent immunoassays contained within a single well. A response for each spot was measured, a single, corresponding local background response was subtracted (refer to example *inset* in **b** that shows the 8×2 grid of 9×9 pixel circles used with ScanAlyze microarray analysis software), the two highest and lowest values (per well) were discarded, and the mean and standard deviation of the remaining four responses were plotted
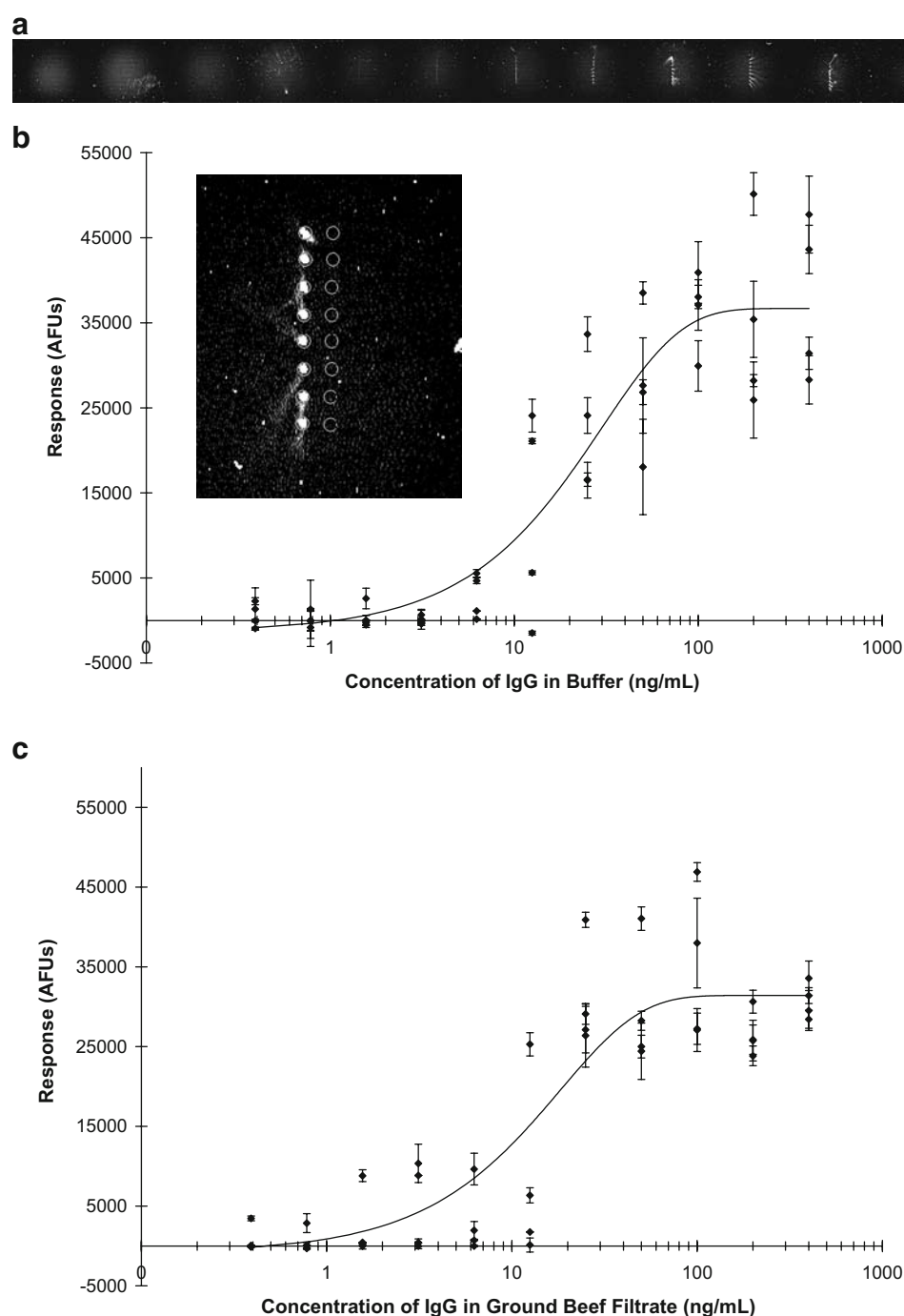
antibody microarray developed in this study. As indicated in Fig. 3, chicken IgG was successfully captured and detected and exhibited an increase in response with concentration that was visually evident in a row of sample wells belonging to a single dilution series in CGBF as shown in Fig. 3a. Though not readily apparent in the semilog plots presented, analysis revealed a relatively small, ca. fivefold, linear dynamic range from ca. 5 to 25 ng/mL that was generally followed by saturation of response at higher concentrations of analyte in either the PBS or CGBF (Fig. 3b and c, respectively). No blank (0 ng/mL IgG samples) response was visually observed or quantitated with the image analysis software (ScanAlyze) employed.

Capture and detection of foodborne pathogenic bacteria

As shown in Fig. 4, visualization (representative images inset in Fig. 4a and b) of antibody microarray detection was
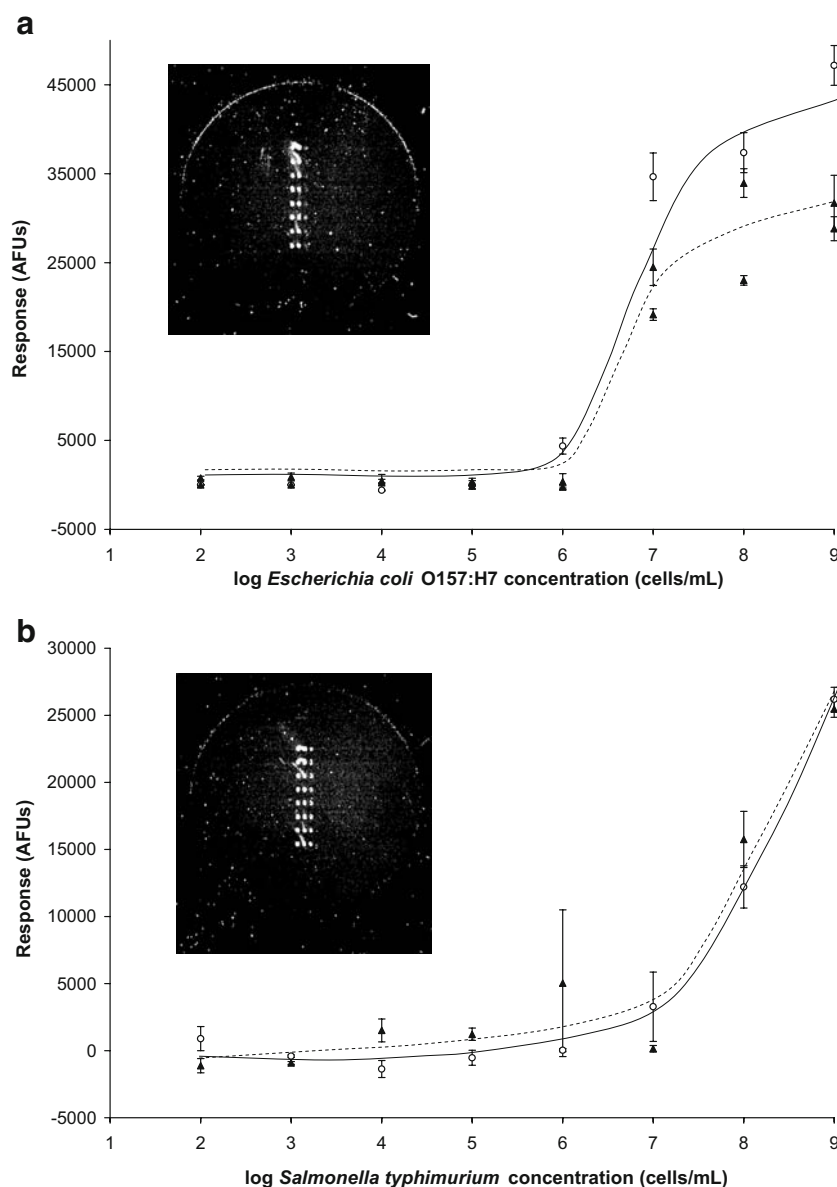
a Response vs. concentration

b Response vs. concentration

Fig. 4 Multiplex detection of *E. coli* O157:H7, *S. typhimurium*, and chicken IgG using fluorescent sandwich immunoassay in microarray format. **a** Response vs. concentration of *E. coli* O157:H7 (1×10^2 to 1×10^9 cells/mL) in buffer (PBS; *solid line/open circles*) or *E. coli* O157:H7 (1×10^2 to 1×10^9 cells/mL) in cultured ground beef filtrate (CGBF; *dotted lines/closed triangles*) that contained *S. typhimurium* (1.0×10^8 cells/mL) and chicken IgG (100 μg/mL). *Inset* is a close-up of a microtiter plate well that contained 1×10^9 cells/mL of *E. coli* O157:H7 (eight spots, *left column* of the array), *S. typhimurium* (eight spots, *right column*), and chicken IgG (eight spots, *middle column*). **b** Response vs. concentration of *S. typhimurium* (1×10^2 to 1×10^9 cells/mL) in buffer (PBS; *solid line/open circles*) or *S. typhimurium* (1×10^2 to 1×10^2 to 1×10^9 cells/mL) in CGBF (*dotted line/closed triangles*) that

contained *E. coli* O157:H7 (1.0×10^8 cells/mL) and chicken IgG (100 μg/mL). *Inset* is a close-up of a microtiter plate well that contained 1×10^9 cells/mL of *S. typhimurium* (eight spots, *right column* of the array), *E. coli* O157:H7 (eight spots, *left column*), and chicken IgG (eight spots, *middle column*). Aliquots of samples in a single dilution series were analyzed once for each varied bacterial analyte (with the exception of *E. coli* O157:H7 in CGBF that was measured in duplicate). As with the analysis of IgG in Fig. 3, each data point represents eight spots or fluorescent immunoassays contained within a single well. As with the Fig. 3 result, responses for each spot were measured, local backgrounds were subtracted, the two highest and lowest values (per well) were discarded, and the mean and standard deviation of the remaining four responses were plotted

straightforward for the bacteria *E. coli* O157:H7 and *S. typhimurium* in PBS and CGBF, albeit at relatively high concentrations (ca. 10^7–10^9 cells/mL). Both *E. coli* O157:H7 (Fig. 4a) and *S. typhimurium* (Fig. 4b) exhibited similar

response curves in PBS and CGBF with a dynamic range from ca. 1×10^6 to 1×10^9 cells/mL and ca. 1×10^7 to 1×10^9 cells/mL, respectively. The presence of a high concentration (100 μg/mL) of chicken IgG as well as a relatively high

concentration (1×10^8 cells/mL) of the bacterial counterpart used in this study left the dynamic range of response essentially unaffected. However, a small decrease in antibody microarray detection response was apparent at the higher levels of *E. coli* O157:H7 concentration in CGBF. As with the IgG analysis, all blank (0 cells/mL of bacteria) responses were negligible as visually assessed or quantified with the image analysis software (ScanAlyze) employed.

Conclusions

In the present study, using static incubation in a 96-multiwell microtiter plate, detection of chicken IgG was realized at levels as low as ca. 5–10 ng/mL in buffer and CGBF. This detection level is comparable to those reported with array flow devices and directly labeled analytes on microarrays [9, 16, 17, 22–24]. Assay times are longer than the Array Biosensor (150 min vs. 30 min), although time per sample is comparable due to the larger number of samples (96 vs. 8). We expect that further optimization will result in lower assay times and detection limits, though the limited dynamic range of the assay may require dilution of samples to attain quantitation.

E. coli O157:H7 and *S. typhimurium* were detected at concentrations of 10^6 and 10^7 cells/mL, respectively. There was only a small decrease in response in the presence of the other analytes or CGBF containing high levels of proteins, lipids, and endogenous microorganisms. These results were generally in line with our past study using a traditional slide-based microarray format [4], which noted similar detection ranges for *E. coli* O157:H7 in pure cultures. Similar detection levels using traditional antibody microarrays for bacterial cells have been reported by others. Rao et al. [25] reported linear detection of *B. globigii* spores from 1.0×10^6 to 1.0×10^8 CFU/mL, and linear detection of MS2 bacteriophage particles from 1.0×10^7 to 1.0×10^9 pfu/mL. Similarly, Belov et al. [26] reported linear detection of whole leukocytes of $1.25{-}10\times10^6$ cells/mL.

A superior detection limit of 6.2×10^4 CFU/mL (in a total assay time of 15 min) was reported by Delehanty and Ligler [22] with a fluorescence immunoassay in microarray format that incorporated a flow system used to detect the bacteria *Bacillus atrophaeus* (formerly *Bacillus globigii*). Using the NRL array biosensor, a biosensor shown to simultaneously be used for the detection of both small toxins and bacterial cells, impressive results for the detection of bacterial cells have been compiled in Ligler et al. [19] and include demonstrated detection of *Campylobacter jejuni* in buffer and foods from 500 to 3,850 CFU/mL by Sapsford et al. [27] as well as demonstrated detection

of *E. coli* O157:H7 in buffer and foods from 5×10^3 to 1×10^4 cells/mL, respectively, by Shriver-Lake et al. [15]. This relatively enhanced detection appeared to be a consequence of elevated cell capture efficiency achieved by flowing bacterial cells past the immobilized capture antibody. Bacterial cells are essentially the same density as water and therefore efficiency of capturing cells at planar surfaces is very poor [4, 28].

Future research will address improvement of microarray printing performance (i.e., attempts to eliminate spot smearing on the unconventional, streptavidin-coated polystyrene microarray substrate by optimizing the capture antibody solution composition as well as fine adjustment of the "z-axis" or up/down robot-controlled pin printing distance to account for potential variations in substrate surface anomalies including the presence of residual solids; the latter maladjustment sometimes led to small spots or lines in between microarrayed spots due to apparent dragging of the pin tip on the substrate), reducing total assay time, and improving detection limit for bacterial cells. Heat treatment of bacterial cells has been demonstrated to improve sensitivity of response [29, 30] and will be a consideration. There is evidence that sample incubation can be considerably reduced since saturation of response for bacterial capture was reported to be maximal after only ca. 10 min of static incubation [4]. Some limits on performance resulted from imaging the arrays through the bottom of the wells. Imaging from the top of the well (with a different scanner) is expected to eliminate background artifacts from debris and scratches on the bottom of the wells and provide greater precision and sensitivity.

A multiwell, multiplex antibody microarray platform for capture and detection of bacterial cells and biomolecules (i.e., chicken IgG) was developed and used to simultaneously detect the bacteria *E. coli* O157:H7 and *S. typhimurium* as well as chicken IgG in inoculated ground beef samples within 2.5 h. Generation of arrays within multiwell plates has previously required access to expensive, high-end spotting robots. The software developed here allows an inexpensive commercial robot to be used for this application, making the multiwell array platform more accessible to researchers. With further development, this platform may be used to rapidly screen large numbers of food samples for a broad range of pathogenic bacteria and toxins.

Appendix
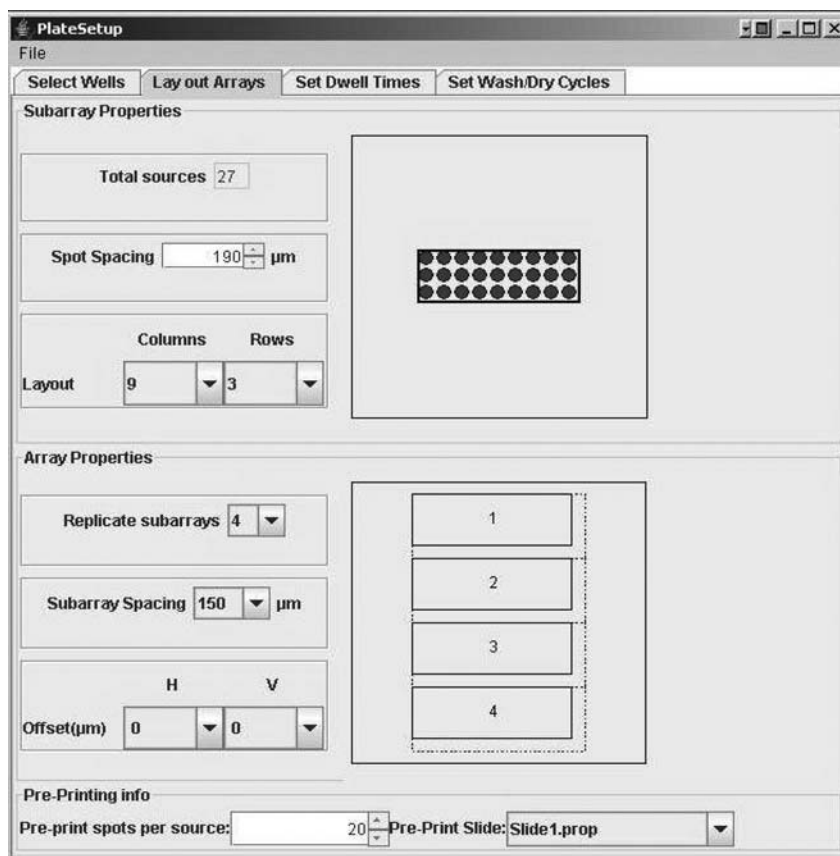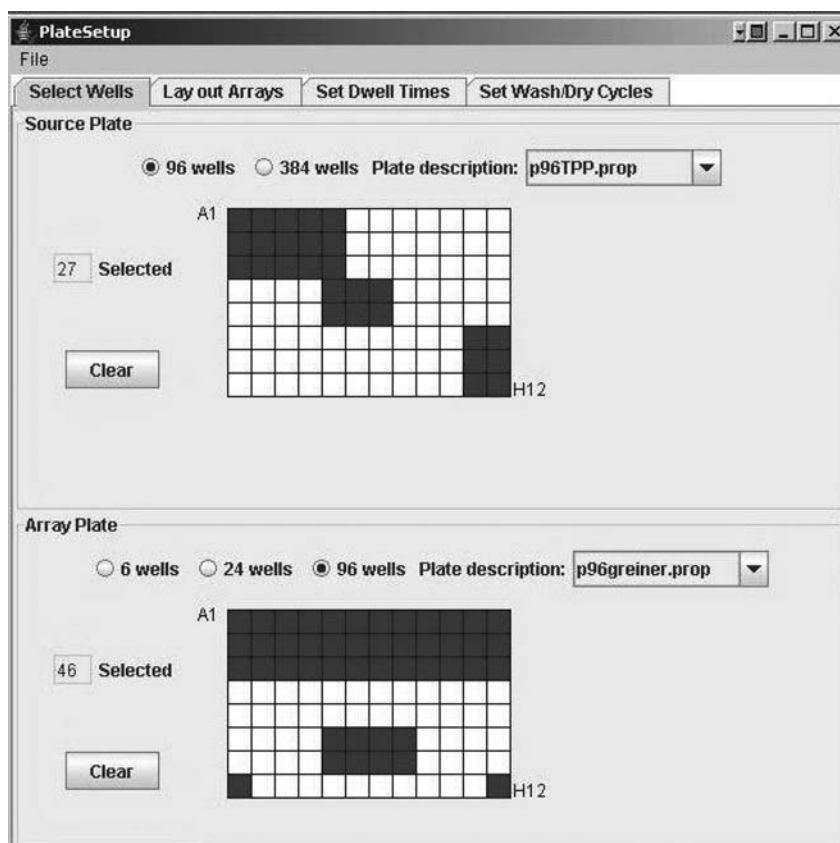
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